NOTES ON SOME BLOOD PARASITES IN REPTILES

BY

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I

Filaria (Mikrofilaria) imperatoris (d'Hérelle and Seidelin, 1909)

Together with F. d'Hérelle I published, in 1909, a short note on two mikrofilariae from the blood of two different snakes from Yucatán, México. We hoped to be able to make further observations on the subject, but so far I have not, for my part, had any opportunity for studying new cases, and probably shall not obtain it soon. I have, therefore, re-examined the specimens from the one case, which belonged to me, and propose now to give a somewhat more detailed description of the parasite. The observation is necessarily incomplete, as I have only had at my disposal dried and stained films, and, moreover, adult filariae were not found. The description and figures may, however, facilitate the identification if some other observer should meet with the same parasite.

The blood films were obtained from a mesenteric artery of a *Boa imperator* which was brought, badly injured but yet with signs of life, to the laboratory. They were fixed in methyl alcohol and stained with Giemsa's stain. As the preparations were only intended to contribute to an investigation of the frequency of haemogregarines they were laid away to be examined later, and thus the opportunity was lost of examining the parasite *in vivo* and of obtaining films for wet fixation.

The parasites were fairly numerous, numbering about seventy in a slide smear. Their general form and structure correspond well to the description and figures of other blood filariae by Annett, Dutton and Elliott (1901), Looss (1905), Manson (1907), and others. The long, slender body has a blunt anterior extremity—the head—and a rapidly tapering tail. The surface of the body shows in some portions a delicate, transverse striation (fig. 1). The head forms a direct prolongation of the body, and is provided with a fine mouth-opening, situated somewhat to the (ventral) side; besides numerous small reddish dots no other definite structures are observed, especially no 'spicule' or 'prepuce.' Beginning at the neck and continued throughout the body are seen larger granules, which have the aspect and staining properties of cell nuclei. Several hundreds of these granules are present, and they are situated in several longitudinal rows which are, however, interrupted at two or three places. The first interruption seems to correspond to the so-called nerve spot, in which no structure is seen; the second is occupied by the 'excretory cell,' an ovoid body containing a clear space, the outlines of which are nearly concentric with those of the 'cell.' The excretory pore is not seen. The third interruption probably contains the 'genital cell,' but such a body cannot be distinguished. The last few granules in the tail are situated in a single row.

The most peculiar feature of these mikrofilariae is that each embryo is situated in an envelope which is generally egg-shaped, but sometimes more oblong. Only in a few cases is the embryo observed free, but then the empty envelope is seen in its immediate vicinity so that the liberation would seem to have taken place artificially, by the spreading of the blood. The envelopes stain a pale blue, and do not show any structure. In their interior the parasites occupy different positions, being sometimes curled up in a spiral (fig. 2), and sometimes nearly straight (fig. 3); between these two extremes all intermediate positions are seen. One cannot help being impressed with the view that individuals like the one depicted in fig. 2 are embryos enclosed in their egg-membranes. On the other hand, the aspect of individuals like fig. 3 resembles considerably that of an ordinary mikrofilaria in its sheath, although the envelope does not adapt itself to the shape of the worm. As a whole the different forms observed correspond closely to the figures given by Manson (1883), as illustrating the transformation of the egg membrane into a sheath, and it would, therefore, seem that our observation supports the view advocated by that authority upon the process, a view which has not been universally accepted. Such huge bodies as the envelopes would, of course, if comparatively rigid, be unable to circulate through the blood-capillaries of man;

how far they might do so in those of a snake, I do not know, since the blood was, as it has been said, taken from a mesenteric artery, a vessel of a large diameter.

The principal dimensions of the worm and its envelope, as measured on dried and fixed films, are as follow (average of various individuals): —

Total length of body, 167 μ.

Width of body, 4 μ.

Distance from anterior extremity to nerve spot, 45 μ.

Distance from anterior extremity to excretory cell, 65 μ.

Distance from anterior extremity to genital spot, 128 μ.

Diameter of granules, 1·3 μ.

Long diameter of excretory cell, 2·4 μ.

Short diameter of excretory cell, 2 μ.

Length of envelope, 115 μ (maximum 142 μ, minimum 86 μ).

Width of envelope, 34 μ.

In the *Boa imperator* neither adult filariae nor embryos seem to have been observed before, but in the *Boa constrictor* three filariae have been described: *Filaria boae constrictoris*, Leidy, 1850; *F. bispinosa*, Diesing, 1851; and *F. mucronata*, Molin, 1858. They are stated to occur 'between the muscles of the ribs and the integument,' 'in cavo abdominis' and 'in cavitate thoracis and vasa majora' respectively. In no case are embryos mentioned.

Filarial embryos in the blood of other reptiles have been briefly described by Rodhain (1906) and mentioned in a footnote by Prowazek (1907).

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II

Haemogregarina imperatoris

In the same blood-smears a haemogregarine was observed which probably represents a new species, since it shows some peculiar morphological features, and, moreover, differs from most other known haemogregarines by the deleterious influence which it exerts upon its host-cells.

The infection was an abundant one, and the elements observed belong evidently to different phases of evolution; but the scarcity of the material at my disposal precludes a full consideration of these questions.

The intracorpuscular parasites which appear least advanced in development are nearly cylindrical bodies with slightly rounded extremities; their length is somewhat less than that of a normal erythrocyte. The protoplasm stains very feebly, and contains only a few reddish granules (Giemsa stain) or no granules at all. The nucleus is generally situated in the central portion of the body, and shows a dense chromatin reticulum, but no definite structural elements can be distinguished. A capsule seems always to enclose the parasites, but it is not always well defined (fig. 16).

In the somewhat larger forms, the granules in the protoplasm have increased both in number and size and, when prominent, stain exactly like chromatin (figs. 17 and 18). The granules show a more or less irregular distribution, and two or three of them may be exceptionally large (fig. 19), but no one can be pointed out with certainty as blepharoplast or other definite structure. Besides the large granules others may be present which are fine, dust-like, and occasionally very numerous. Granules are as a rule not mentioned in the description of haemogregarines, but those here described evidently correspond to the volutin-granules of Reichenow (1910). The protoplasm shows, moreover, in the advanced stages, a diffuse colouring, pale blue or sometimes more pink. The nucleus is similar to that of the smaller forms, but it is more often eccentrically situated, and there can frequently be seen one or two nucleoli in its interior (fig. 20). The chromatin of the nucleus sometimes forms well delimited large granules, which are united only by delicate filaments (fig. 21), and in a few cases a formation of definite chromosomes seems to have taken place, eight such being distributed in two rows of four each (fig. 22); some more advanced stages of division are also observed (fig. 23). The capsule is in the larger forms fairly well defined, and its extremities are often delimited from the central portion by delicate lines which are stained red (figs. 17 and 25); such lines are considered constant by Sambon (1908-9), but they are seldom mentioned or figured by other authors. I have seen them frequently in other haemogregarines also, but by no means constantly, and never so sharply defined as they appear in Sambon's somewhat schematic figures. If this author is right in describing them as lines of cleavage, it is readily understood that they may be inconstant.

In their most advanced stages the intracorpuscular elements attain almost the dimensions of a normal erythrocyte (figs. 24 and 25). They still conform essentially to the description already given, but in some individuals one extremity is very slender and bent against the body in an acute angle (fig. 26).

A number of parasites belong to a different type. They are long and slender, with one blunt extremity, and the other slowly tapering. Being doubled up they easily find room in the erythrocytes, although they are much longer than their host-cells; sometimes the extremity of the tail is again bent in a sharp angle against the body. The protoplasm stains dark blue or violet, and contains a number of fine and occasionally a few coarse granules. The nucleus is situated near the bend, in the anterior half of the body; it is approximately quadrangular, and occupies the whole of the width of the body. It is sharply limited and deeply stained (fig. 27). The long forms are in this case always intracorpuscular; in the blood of other snakes, however, I have repeatedly seen them free in preparations which were taken from the living animal and immediately fixed. I emphasize this, because Flu (1910), Reichenow (1910), and others state that these elements are always intracorpuscular, normally, and become free only under abnormal conditions, as when the blood is being preserved outside the body.

These long elements have, according to Lutz (1901), and most other authors, no direct connection with the more oval forms; the former are derived from mikrozoits, the latter from makrozoits.

Reichenow (1910), however, asserts that the long forms are at a later stage of the evolution transformed into oval bodies, the extremities being drawn in when the parasites are preparing to divide. All schizonts are said to undergo such a transformation, and it would seem not at all unlikely that some of our larger forms belong to this group, the significance, especially of the large oval elements with short slender tail (fig. 26), becoming intelligible if they are considered as transitional stages. It is, of course, possible to adopt this view with regard to the larger forms only; the small oval parasites must have a different origin, and would, according to Reichenow, represent gametes, but this view does not agree with the presence of mitotic figures in several of them. The distinction between male, female and indifferent haemogregarines which is made by many authors depends, however, to a large extent on analogy, and cannot be too carefully considered before being accepted. In the present case no such differentiation can with certainty be made.

In this case no small parasites were seen free in the blood-plasma, except the one depicted in fig. 28, which appears to have just escaped from its host-cell. An erythrocyte is also shown in fig. 29, which evidently has harboured a parasite. The possibility cannot be denied that these appearances may have been produced artificially. Another very interesting individual is seen in fig. 30; it consists of a large body with faintly blue-stained protoplasm and numerous chromatin-granules, whilst no well defined nucleus is seen. At one extremity of this body is seen another structure, much smaller and very slender, half inside, half outside the larger one; the smaller element is wholly chromatin-stained. The whole appearance suggests strongly the penetration of a mikrogamete into a makrogamete. The possibility of conjugation taking place in the body of the snake is admitted by Hartmann and Chagas (1910). So far very little is known about the sexual phase of the snake-haemogregarines and about the allied subject of their transmission; with regard to *Haemogregarina stepanowi* of tortoises, Reichenow (1910) has shown that the sexual phase is found in a leech.

A few words should now be said about the effect of the haemogregarines on the erythrocytes. In the case of the smaller parasites their host-cells do not show any important alterations, excepting a slight increase in size and a dislocation of the nucleus. Corresponding to the somewhat larger forms, the erythrocytes are not only enlarged, but often differentiated into a pale peripheral portion and a more deeply staining zone surrounding the parasite. The corpuscles which harbour the most advanced stages of parasites are of enormous size, their length being about twice the normal; they have apparently lost all their haemoglobin, and have become mere shadows, the outlines of which it may be difficult to distinguish. In a few instances numerous fine red granules are seen in the decolourized erythrocytes, perhaps representing remains of the broken-up haemoglobin, but closely resembling the fine granules of the parasites (fig. 25). The nuclei become disfigured by compression and their structure more dense, but no fragmentation is observed, as has been described by Marceau (1901) and others. This deleterious influence on the erythrocytes constitutes a considerable difference from what is the case in most haemogregarine-infections, in which as a rule no alteration of the host-cells is observed, or (in the case of *Karyolysus* sp.) only of the nucleus. Prowazek (1907), however, gives figures (from lizards) very similar to our own.

Besides these parasites I must briefly mention other bodies which are of interest, as they may easily be mistaken for free forms of haemogregarines. In fact, I am not absolutely sure that they are normal blood elements, but there is a great probability in favour of their being so. I refer to the bodies depicted in figs. 4-11 on Plate XIV, which are characterized by a large, well-staining nucleus, a nearly unstained protoplasm, sometimes very scarce, and a well-defined red border-line. Two nuclei may be present, as in fig. 11, but no mitotic figures are seen. These corpuscles are similar to haemogregarine-merozoites, as figured by Reichenow (1910) and others, but on the other hand they bear a striking resemblance to some figures which are given by Werzberg (1910). This author has found such bodies in the blood of *Tropidonotus natrix*, and in *Chamaeleon* sp., and is inclined to consider them as thrombocytes, though he leaves room open for doubt. On examining, here in England, the blood of a *Tropidonotus natrix*, I have also found similar cells, and this circumstance, of course, confirms the identity of those seen by Werzberg and by myself. One considerable difference seems, however, to exist, namely, in the staining of the nucleus. The figures of Werzberg show a very pale nucleus, whilst my preparations, both from *Tr. natrix* and especially from *Boa imperator*, show a deep staining of the nucleus. This may be due, perhaps, to the different methods of staining, Werzberg having used Pappenheim's so-called Unna-Ziehl stain, and I the Giemsa stain; but as other nuclei, especially of the erythrocytes, in Werzberg's figures are deeply coloured, the explanation is not quite satisfactory. However, it may be admitted that the bodies are probably of the same nature, and are constituents of the blood; this is so much the more likely, as, in *Tr. natrix* they do not show any movements in fresh preparations, as haemogregarines probably would do. It then remains to consider what their real nature may be. In both cases the border lines stain red; this seems by the Unna-Ziehl stain to indicate a relationship to the erythrocytes, as the cytoplasm of these elements takes the same colour. But in my specimens the red is not like the eosin colour of the erythrocytes, it resembles much more the azur-eosin stained chromatin. By other methods, it does not give the reaction of either. It does not stain like chromatin with iron-haematein, nor does it take up eosin, like haemoglobin; in both cases it remains unstained. Moreover, it retains its colour badly; in Giemsa-preparations it is only visible in dry smears when no differentiation is employed. Subsequent to the differentiation with acetone which becomes necessary when the 'wet method' is used, no red colour is to be found. By this differentiation the nuclei of these bodies are also often to a large extent decolourized, though all the surrounding erythrocyte-nuclei are deeply stained. In fresh preparations it is in most cases difficult to observe more than the nuclei of these cells, but occasionally the border-line appears quite sharp, and the protoplasm very slightly granular. Moreover, their protoplasm appears to be very fragile, or at least very plastic, since the bodies in the stained preparation present very irregular and varied forms. The border-line is evidently but loosely connected with the cytoplasm, since it often becomes separated from its periphery and takes on different positions, sometimes so peculiar that a certain resemblance to a flagellum is produced (figs. 4, 7 and 8). How far these characters are artificially produced by the preparation of the films it is difficult to say, since an exact observation of the bodies was not possible in the specimens which were examined in the fresh state or treated by 'wet' fixation and staining, as above-mentioned.

Similar bodies, but without a definite border-line, were found in the blood of other snakes in Yucatán; haemogregarines were also present. Only in the case of the *Tropidonotus natrix* have no intracorpuscular parasites been detected. No relationship is apparent to other blood elements; at present these bodies must be classified as 'thrombocytes,' a special kind of cells, probably corresponding to the platelets of higher animals.

Another peculiar kind of cell, the significance of which has not been determined, is represented in figure 12. They give at first view the impression of leucocytes, but a more close inspection shows them to be enclosed in a large, faintly eosin-stained protoplasmic body, similar to that of an altered erythrocyte. Their outlines are nearly circular, they have a granular, dark-staining protoplasm, and an eccentrically situated dense nucleus. It cannot be determined if these bodies are of a parasitic nature, or if they represent a stage of evolution of certain blood-elements.

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III

Amoeboid parasites in the blood-plasma of a Lizard

In the blood of a lizard (*Lacerta* sp.) from Yucatán, which has not been identified, I observed amoeboid parasites in the plasma, while no intracorpuscular forms were seen. Similar known parasites are, at least in some stages of their development, intracorpuscular. Individuals of very different sizes and shapes were observed but all seem to belong to the same kind of organism. The differences correspond probably to different evolutionary phases, although of course the results cannot be entirely relied upon to show exact details considering the technique employed (dry smears, methylalcohol fixation, Giemsa stain). I shall consequently not attempt a detailed description, much less classification, but only shortly state with the aid of illustrations what I saw.

A large number of minute bodies are seen with a fine chromatin dot and a blue protoplasm which is occasionally solid, but as a rule forms a more or less delicate ring around a comparatively large vacuole (fig. 31).

From this stage all subsequent gradations in size are met with (figs. 32-40) until the largest forms which consist of a protoplasmic body the size of which approximates to that of an erythrocyte, and which shows a granular structure and contains numerous small vacuoles and very often one or two larger ones. In most cases one nucleus is present; it is always small and consists of a central darker, and a peripheral less intensely stained portion; often a second, still smaller chromatin-stained element is also present at a considerable distance from the nucleus (fig. 36), and occasionally two nuclei of about equal size are seen (fig. 33); when the latter is the case the cytoplasm shows a certain symmetry and we possibly have to do with a divisional phenomenon. This would also agree with the regular. rounded shape of such elements, but, on the other hand, they are not of particularly large dimensions. In several instances no definite nucleus is present, but several small chromatin-granules. Many of the parasites show pseudopodia in different numbers and of different shapes. The fixation has probably given them a more rigid aspect than they would have had if examined in the living state.

Forms like those shown in figs. 41 and 42 may possibly be distorted amoebae, although the possibility of different kinds of parasites being present must also be considered.

It is remarkable that this extracorpuscular parasite appears to have a more pronounced effect upon the erythrocytes than intracorpuscular parasites often have. Many erythrocytes are metachromatic and vacuolated, and in places where they are closely grouped together the plasma shows a faint eosin staining, a sign of haemolysis having taken place. Related to this phenomenon is probably another which was also observed in this case, namely, the presence of numerous pigmented leucocytes, as shown in the figs. 13, 14 and 15. I can find no mention of these in works on haemogregarines or other intracorpuscular parasites of reptiles. Nor have I observed them in such infections; besides in the case here reported I have only met with pigmented leucocytes once in a lizard, but I have no notes on the presence of parasites in that case. The aspect of the pigment-granules resembles that of malarial pigment, but no statement as to its nature can be made. One would naturally be inclined to connect the pigment with the destruction of haemoglobin; it may be pointed out, however, that Downey (1910), who recently has mentioned the existence of similar pigment in phagocytes from the lympho-renal tissue of a fish (*Polyodon spathula*) describes in detail the transformation, not of the cytoplasm of the erythrocytes, but of their nuclei, into pigment. Here is evidently a point which, for general pathological reasons, deserves further investigation.

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DESCRIPTION OF PLATES

All figures have been drawn with Abbé's apparatus, using Zeiss's 3 mm. apochromatic objective and, in the figs. 4-42, compensating ocular 12; for fig. 1 compensating ocular 8, and for figs. 2 and 3 compensating ocular 4 has been used. The magnifications are respectively 1300, 850 and 450 linear. Most of the figures have been executed by Mrs. Margrethe Seidelin.

Plate XIV

Figs. 1-3. Filaria-embryos.

Figs. 4-11. Various forms of 'Thrombocytes.'

Fig. 12. Leucocyte-resembling cell in large protoplasmic body; to the left a lymphocyte.

Figs. 1-12 from the blood of *Boa imperator*.

Fig. 13 Pigmented mononuclear leucocyte.

Fig. 14. Pigmented leucocyte with two nuclei.

Fig. 15. Pigmented and unpigmented leucocyte fusing together

Figs. 13-15 from the blood of *Lacerta* sp.

Plate XIV

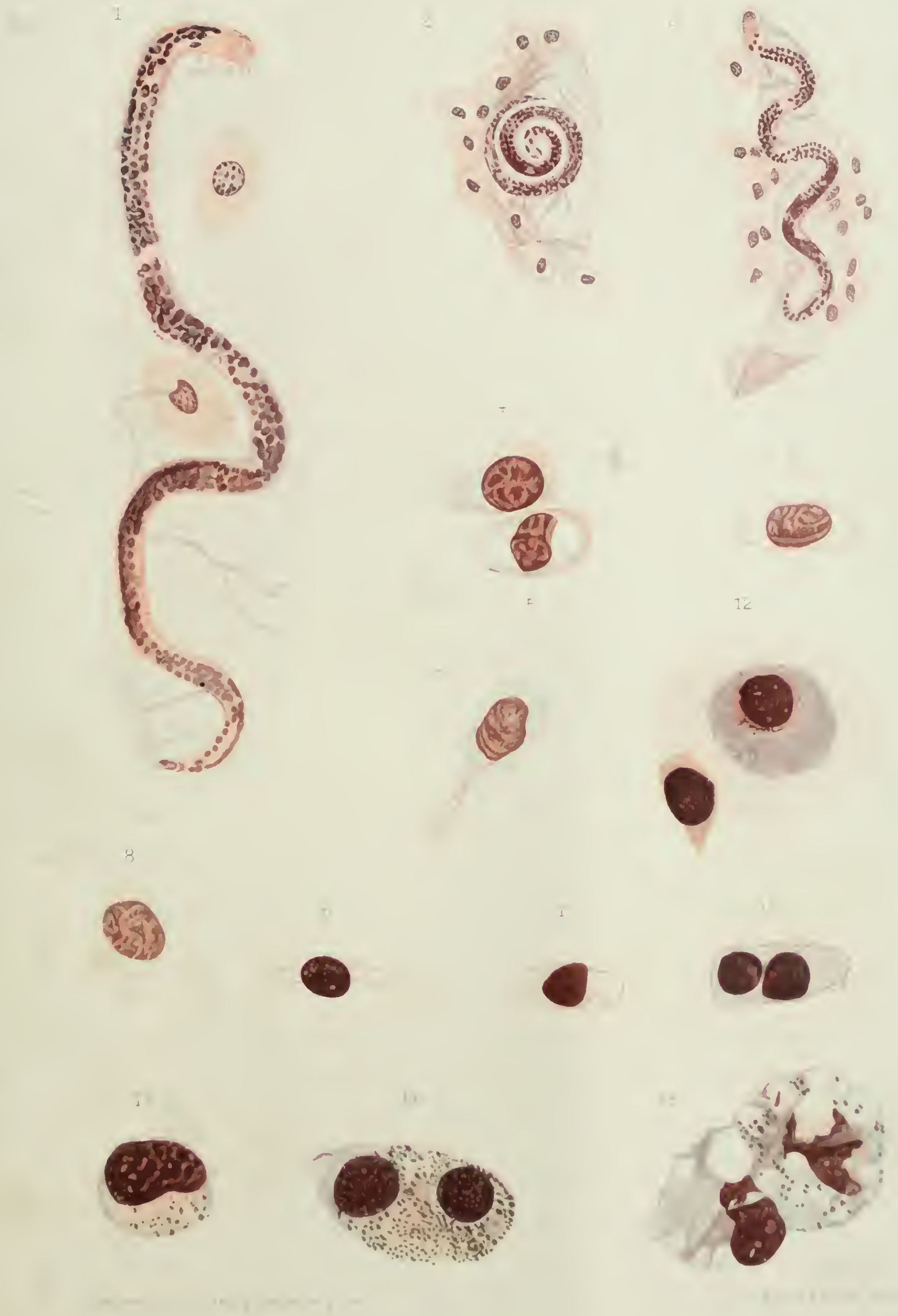

Plate XV

Figs. 16-30. Haemogregarines in blood of *Boa imperator.*

Figs. 16 and 17. Young forms with pale protoplasm and few granules.

Figs. 18-20. Larger forms with more deeply stained protoplasm which contain numerous large granules; in fig. 18 three such granules are very prominent.

Fig. 21. The chromatin in the nucleus forms regular granules.

Figs. 22 and 23. Mitotic figures.

Figs. 24 and 25. Large forms in enlarged and decolourized erythrocytes; in fig. 25 and 26, especially in the first, numerous fine red granules are seen in the cytoplasm of the erythrocyte.

Fig. 27. Long, slender form.

Fig. 28. Parasite escaping from host-cell.

Fig. 29. Empty host-cell.

Fig. 30. Large free form; conjugation ?

In Figs. 19, 20, 25 and 26 cleavage lines are seen.

Figs. 31-42. Parasites from the blood of *Lacerta* sp.

In Figs. 35, 39 and 40 pseudopodia are seen. Figs. 41 and 42 distorted amoebae or flagellata.

Plate XV